Chloroform (CASRN 67-66-3)

view QuickView

List of IRIS Substances

Select a Substance go

Full IRIS Summary QuickView

MAIN CONTENTS

Reference Dose for Chronic Oral Exposure (RfD) go

Note: A TOXICOLOGICAL REVIEW is available for this chemical in Adobe* PDF format (112 Pages, 760 Kbytes). Similar documents can be found in the List of Available IRIS Toxicological Reviews.

Quantitative Dose-Response Modeling, which accompanies the toxicological review, is available in the attached electronic Zip file (193 K). Click on this link, and then click on "Save File."

Links to specific pages in the toxicological review are available throughout this summary. To utilize this feature, your Web browser and Adobe program must be configured properly so the PDF displays within the browser window. If your browser and Adobe program need configuration, please go to the IRIS Help page for instructions.

0025

Chloroform; CASRN 67-66-3; 10/19/01

Health assessment information on a chemical substance is included in IRIS only after a comprehensive review of chronic toxicity data by U.S. EPA health scientists from several Program Offices and the Office of Research and Development. The summaries presented in Sections I and II represent a consensus reached in the review process. Background information and explanations of the methods used to derive the values given in IRIS are provided in the Background Documents.

STATUS OF DATA FOR Chloroform

File First On-Line 01/31/1987

Category (section)	Status	Last Revised
Oral RfD Assessment (I.A.)	on-line	10/19/01
Inhalation RfC Assessment (I.B.)	not available	
Carcinogenicity Assessment (II.)	on-line	10/19/01

_I. Chronic Health Hazard Assessments for Noncarcinogenic Effects

_I.A. Reference Dose for Chronic Oral Exposure (RfD)

Chloroform
CASRN -- 67-66-3
Last Revised -- 10/19/01

The oral Reference Dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg/day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. Please refer to the Background Document for an elaboration of these concepts. RfDs can also be derived for the noncarcinogenic health effects of substances that are also carcinogens. Therefore, it is essential to refer to other sources of information concerning the carcinogenicity of this substance. If the U.S. EPA has evaluated this substance for potential human carcinogenicity, a summary of that evaluation will be contained in Section II of this file.

Quantitative Estimate of Carcinogenic Risk from Oral Exposure

- Summary of Risk Estimates
- Dose-Response Data
- Additional Comments
- Discussion of Confidence

Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

- Summary of Risk Estimates
- Dose-Response Data
- Additional Comments
- Discussion of Confidence

EPA Documentation, Review and, Contacts

- Bibliography
- Revision History
- Synonyms

__I.A.1. Oral RfD Summary

Traditional Approach

For comparison purposes, an RfD was developed using the traditional NOAEL/LOAEL approach. The results of this method are provided below. This is the same approach and RfD result reported on IRIS (01/13/87).

Critical Effect	Experimental Doses*	UF	MF	RfD
Moderate/marked fatty cyst formation in the liver and elevated SGPT Dog, chronic oral bioassay Heywood et al., 1979	NOAEL: none LOAEL: 15 mg/kg/day (converted to 12.9 mg/kg/day)	1,000	1	0.01 (mg/kg/day)

*Conversion Factors and Assumptions -- 15 mg/kg/day × 6 days/7 days = 12.9 mg/kg/day.

__I.A.2. Principal and Supporting Studies (Oral RfD)

Heywood, R; Sortwell, RJ; Noel, PRB; et al. (1979) Safety evaluation of toothpaste containing chloroform: III. Long-term study in beagle dogs. J Environ Pathol Toxicol 2:835-851.

Heywood et al. (1979) exposed groups of eight male and eight female beagle dogs to doses of 15 or 30 mg chloroform/kg/day. The chemical was given orally in a toothpaste base in gelatin capsules, 6 days/week for 7.5 years. This was followed by a 20- to 24-week recovery period. Eight dogs of each sex served as an untreated group and a final group of 16 dogs (8/sex) received an alternative nonchloroform toothpaste (vehicle control). Four male dogs (one each from the low- and high-dose chloroform groups, the vehicle control group, and the untreated control group) and seven female dogs (four from the vehicle control group and three from the untreated control group) died during the study. In the low-dose group, levels of serum glutamate-pyruvate transaminase (SGPT, also known as alanine aminotransferase) were increased by an average of about 40% compared with control, with the effects

being statistically significant from week 130 through week 364. In the high-dose group, SGPT levels tended to average about twice those in the control group, and the differences were statistically significant from week 6 throughout treatment. After 14 weeks of recovery, SGPT levels remained significantly increased in the high-dose group, but not in the low-dose group, when compared with the controls. After 19 weeks of recovery, SGPT levels were not significantly increased in either treated group when compared with the controls. The authors concluded that the increases in SGPT levels were likely the result of minimal liver damage. Serum alkaline phosphatase (SAP) and SGPT levels were also moderately increased (not statistically significant) in the treated dogs at the end of the treatment period when compared with the controls. Microscopic examinations were conducted on the major organs. The most prominent microscopic effect observed in the liver was the presence of "fatty cysts," which were described as aggregations of vacuolated histiocytes. The fatty cysts were observed in the control and treated dogs, but were larger and more numerous (i.e., higher incidence of cysts rated as "moderate or marked," as opposed to "occasional or minimal") in the treated dogs than in the control dogs at both doses. The prevalence of moderate or marked fatty cysts was 1/27 in control animals, 9/15 in low dose animals, and 13/15 in high dose animals. Nodules of altered hepatocytes were observed in both treated and control animals, and therefore were not considered related to treatment. No other treatment-related nonneoplastic or neoplastic lesions were reported for the liver, gall bladder, cardiovascular system, reproductive system, or urinary system. A NOAEL was not identified in this study. However, a LOAEL of 15 mg/kg/day was identified, based on elevated SGPT levels and increased incidence and severity of fatty cysts (U.S. EPA, 1998a).

Benchmark Dose (BMD) Approach

Selection of Data Sets for Modeling

The following data sets were selected for BMD modeling:

- Incidence of fatty cysts in liver and SGPT levels of dogs (Heywood et al., 1979)
- Histological evidence of renal cytotoxicity in male rats exposed via drinking water (Hard et al., 2000)
- Increased labeling index in kidney of female mice exposed via drinking water (Larson et al., 1994b)
- Increased labeling index in liver of female rats exposed via gavage in corn oil (Larson et al., 1995b)

These studies were chosen because they all provide quantitative dose-response data for sensitive indicators of chloroform toxicity.

BMD Modeling of Selected Data Sets

The detailed results of the BMD model fitting are presented in Appendix B of the Toxicological Review of Chloroform. Within a data set, the preferred model was selected based on the quality of the model fit to the data.

As seen, the kidney LI data set from Larson et al. (1994b) could not be adequately described by any of the continuous models. This is because even though the response was statistically significant, the magnitude of the response was small in comparison to normal variability, and the data did not form a smooth dose-response relationship (tending to first increase and then decrease as dose increased). The liver and kidney LI data sets from Larson et al. (1995b) were reasonably well fit by the Hill

equation, with BMD values of 64-75 mg/kg/day. However, the software was not able to estimate a benchmark dose limit (BMDL) value in either case. The data sets from the studies by Hard et al. (2000) and by Heywood et al. (1979) were adequately fit by one or more of the dichotomous models, with the best fit being given by the log-logistic and the quantal-linear models, respectively. The preferred BMD of 70 mg/kg/day based on the renal cytotoxicity data of Hard et al. (2000) is similar to the BMD values derived for the LI data from Larson et al (1995b), but is significantly higher than the preferred BMD based on the incidence of fatty cysts in dogs (1.7 mg/kg/day) reported by Heywood et al. (1979). The basis for this marked difference in BMD between studies is not known, but the data suggest that liver toxicity in the dog is a more sensitive endpoint of chloroform toxicity than renal or liver cytotoxicity in rodents.

Calculation of the BMD-Based RfD

Critical Effect	Experimental Doses*	UF	MF	RfD
Moderate/marked fatty cyst formation in the liver and elevated SGPT Dog, chronic oral bioassay Heywood et al., 1979	$BMDL_{10}$: 1.2 mg/kg/day (converted to 1.0 mg/kg/day)	100	1	0.01 (mg/kg/day)

The $BMDL_{10}$ provided in the table represents the 95% confidence lower bound on the dose associated with a 10% extra risk based on the prevalence of animals with moderate to marked fatty cysts in liver and elevated SGPT. The value of the $BMDL_{10}$ was calculated from the data of Heywood et al. (1979) using EPA's BMDS software Version 1.2. The value derived from the BMD modeling (1.2 mg/kg/day) was adjusted by a factor of 6/7 to account for exposure 6 days per week.

__I.A.3. Uncertainty and Modifying Factors (Oral RfD)

UF = 100

In the benchmark dose approach, an uncertainty factor (UF) of 10 was used to account for interspecies extrapolation, and a UF of 10 was used to protect sensitive subpopulations. In the NOAEL/LOAEL approach, an additional factor of 10 was used to account for extrapolation from a LOAEL to a NOAEL (total UF = 1,000). No additional factors were required to account for extrapolation from short term to long term (the study duration was 7.5 years) or to account for limitations in the database.

MF = 1

No additional modifying factors (MFs) were considered necessary because there are no substantial concerns or limitations in the derivation of the RfD that are not accounted for in the UFs described above.

__I.A.4. Additional Studies/Comments (Oral RfD)

In general, the NOAEL/LOAEL approach for derivation of an RfD is subject to a number of limitations, most of which are addressed by use of the BMD approach (U.S. EPA, 1995). Thus, the RfD based on the BMD approach is generally preferred,

unless there are insufficient dose-response data to support derivation of a reliable BMD.

In this case, the dose-response data set from the critical study (Heywood et al., 1979) is composed of only two doses plus a control group. This is considered to be a limitation, as the shape of the dose-response curve is difficult to define with only three values, especially when the lowest dose yields a response that is well above the benchmark response. Nevertheless, the data do yield curve fits of adequate quality, so the results of the BMD approach are considered preferable to the NOAEL/LOAEL approach.

Note that, in this particular case, the two approaches (NOAEL/LOAEL and benchmark) yield equal RfD values. This is consistent, albeit coincidental, with the results from the default LOAEL/NOAEL method.

Many other studies in animals support the conclusion that the liver and/or the kidney are the key target organs for chloroform-induced toxicity. Most of these studies have been performed in rats and mice, and most yield LOAEL values that are substantially higher than those observed in dogs.

In a study conducted by Palmer et al. (1979), in which rats were administered daily oral doses of 60 mg chloroform/kg/day in a toothpaste vehicle, treatment-related effects included a decrease in plasma but not erythrocyte, cholinesterase in females, a decrease in liver weight in females, and a marginal but consistent and progressive retardation in weight gain in both sexes. The authors stated that although minor histological changes in the liver were noted, there was no evidence of severe fatty infiltration, fibrosis, or bile duct abnormalities in the livers of treated animals. The authors concluded that there was no evidence of treatment-related toxic effects in the liver. However, the "minor histopathological" changes in the liver were not described and the presence of any fatty infiltration that would be designated as less than severe was not reported. Therefore, these results could not be compared to those reported in the dog study. The LOAEL for this study was 60 mg/kg/day.

A slight (2%–3% vs. 7%–8%) increase in moderate to severe fatty degeneration of the liver was seen in ICI mice given 60 mg but not 17 mg chloroform/kg/day in a toothpaste vehicle for 80 weeks (Roe et al., 1979). However, no effects were evident when the incidences of fatty and nonfatty liver degeneration were combined in the ICI or three other mice strains. No other noncancer effects attributable to chloroform were noted. A NOAEL of 17 mg/kg/day and a LOAEL of 60 mg/kg/day were identified from this study.

No treatment-related noncancer effects were noted in rats administered chloroform in drinking water for 23 months at time-weighted average doses up to 160 mg/kg/day (Jorgenson et al., 1982, 1985). However, subsequent review of the histopathology slides from this study revealed evidence that chloroform produced a moderate to low level of renal proximal tubule injury associated with cell turnover indicative of cytotoxicity (Hard et al., 2000). These changes were noted in the high-dose (160 mg/kg) group males as early as 12 months but were increased in grade by 18 months. Similar changes were found in the mid-dose males (81 mg/kg), although at a lower grade, in the 18-month and 2-year dose groups. These changes were not seen in controls or the low-dose group. Therefore, the identified NOAEL for noncancer effects for this study is 38 mg chloroform/kg/day, with the LOAEL at 81 mg/kg/day.

In mice exposed to chloroform in drinking water, mortality within the first 3 weeks was significantly increased in the two highest dose groups, 130 and 263 mg/kg/day, but was comparable with controls after that time (Jorgenson et al., 1982). Early mortality and behavioral effects (e.g., lassitude, lack of vigor) were apparently related to reduced water consumption among some treated mice in the two highest dose groups. A significant increase in liver fat in mice was noted at doses of 65 mg/kg/day

and higher at 3 months, but only at doses of 130 and 263 mg/kg/day by 6 months. Liver fat content was not reported for any later time points or at terminal sacrifice; therefore, the relevance of this observation as an adverse effect rather than an adaptive response could not be assessed. No increased incidence of liver tumors was reported, and the presence or absence of nonneoplastic histopathological alterations was not described. These data indicate that doses of 130 to 263 mg/kg/day may produce adverse effects in mice; however, these effects may be secondary to decreased water consumption.

Reproductive/developmental toxicity studies were also considered in the selection of the critical study/effect for the reference dose in the event the fetus represented a more sensitive population. These included studies in rats (Thompson et al., 1974), in rabbits (Thompson et al., 1974), and in mice (NTP, 1988). In the developmental studies in rabbits and rats, no treatment-related effects were noted when chloroform was administered by gavage in corn oil during gestation at doses of 50 mg/kg/day or less (Thompson et al., 1974). In the rabbit study, a clear dose-response was absent and the effects noted in offspring of dams administered chloroform at doses up to 50 mg/kg/day (the highest dose tested) on days 6 to 18 of gestation were not considered to be treatment-related (Thompson et al., 1974). In rats, the only effect noted was a significant reduction in fetal weight found only in offspring of dams given chloroform at the highest dose tested, 126 mg/kg/day, on days 6 to 15 of gestation (Thompson et al., 1974). No fetal effects attributed to chloroform treatment were noted in this rat study for the lower dose groups (up to 50 mg/kg/day during gestation). A NOAEL of 50 mg/kg/day was identified for both studies.

In a two-generation reproductive study in mice, no significant effects were seen in any reproductive parameter assessed in either the parental or the F_1 generations at doses up to 41 mg/kg/day administered by gavage in corn oil (NTP, 1988). Systemic toxicity was not evaluated in the parental generation. However, increased liver weights and liver lesions, described as mild to moderate degeneration of centrilobular hepatocytes accompanied by single-cell necrosis, were noted in F_1 females, but not males, exposed both in utero and postnatally at a dose of 41 mg/kg/day. Postnatal exposure in the F_1 generation began at postnatal day 22 and continued until the birth of the F_2 generation (mice were mated at 64 to 84 days of age). The F_1 offspring in the two lower dose groups, 6.6 and 16 mg/kg/day, were not evaluated histopathologically; therefore, no NOAEL or LOAEL could be definitively established for this study. A dose of 41 mg/kg/day may represent the LOAEL; however, the amount of in utero exposure was not estimated, nor was the contribution of in utero exposure to liver toxicity assessed. Because quantitative data were available only for the control and high-dose groups, the study was not selected for benchmark modeling.

In the reproductive/developmental studies, both maternal toxicity and effects on the fetus or offspring occurred at doses higher than those that produced evidence of liver toxicity in the dog study. Therefore, these were not used as the critical study for derivation of the RfD. ***For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).***

__I.A.5. Confidence in the Oral RfD

Study -- Medium
Database -- Medium
RfD -- Medium

The overall confidence in this RfD assessment is medium. The database on noncancer effects in animals is extensive, and data are adequate to derive reliable

dose-response curves for key endpoints. Confidence is not rated higher because data in humans are limited, and extrapolation from animals to humans (with an attendant uncertainty factor of 10) is required.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

__I.A.6. EPA Documentation and Review of the Oral RfD

Source Document -- U.S. EPA, 2001

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A record of these comments is included as an appendix to U.S. EPA (2001). ***To review this appendix, exit to the toxicological review, Appendix A, External Peer Review -- Summary of Comments and Disposition (PDF).***

Other EPA Documentation -- U.S. EPA, 1994, 1997, 1998a-c, 2001

Agency Consensus Date -- 7/27/2001

__I.A.7. EPA Contacts (Oral RfD)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

Back to top

_I.B. Reference Concentration for Chronic Inhalation Exposure (RfC)

(Not available. To be developed)

Back to top

_II. Carcinogenicity Assessment for Lifetime Exposure

Chloroform
CASRN -- 67-66-3
Last Revised -- 10/19/01

Section II provides information on three aspects of the carcinogenic assessment for the substance in question; the weight-of-evidence judgment of the likelihood that the substance is a human carcinogen, and quantitative estimates of risk from oral exposure and from inhalation exposure. The quantitative risk estimates are presented in three ways. The slope factor is the result of application of a low-dose extrapolation procedure and is presented as the risk per (mg/kg)/day. The unit risk is the quantitative estimate in terms of either risk per µg/L drinking water or risk per µg/cu.m air breathed. The third form in which risk is presented is a concentration of the chemical in drinking water or air associated with cancer risks of 1 in 10,000, 1 in 100,000, or 1 in 1,000,000. The rationale and methods used to develop the carcinogenicity information in IRIS are described in The Risk Assessment Guidelines

of 1986 (EPA/600/8-87/045) and in the IRIS Background Document. IRIS summaries developed since the publication of EPA's more recent Proposed Guidelines for Carcinogen Risk Assessment also utilize those Guidelines where indicated (Federal Register 61(79):17960-18011, April 23, 1996). Users are referred to Section I of this IRIS file for information on long-term toxic effects other than carcinogenicity.

_II.A. Evidence for Human Carcinogenicity

__II.A.1. Weight-of-Evidence Characterization

Under the 1986 U.S. EPA Guidelines for Carcinogen Risk Assessment, chloroform has been classified as Group B2, *probable human carcinogen*, based on "sufficient evidence" of carcinogenicity in animals (U.S. EPA, 1998a).

Under the Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996; U.S. EPA, 1999), chloroform is *likely to be carcinogenic to humans by all routes of exposure* under high-exposure conditions that lead to cytotoxicity and regenerative hyperplasia in susceptible tissues (U.S. EPA, 1998a,b). Chloroform is *not likely to be carcinogenic to humans by any route of exposure* under exposure conditions that do not cause cytotoxicity and cell regeneration. This weight-of-evidence conclusion is based on: 1) observations in animals exposed by both oral and inhalation pathways which indicate that sustained or repeated cytotoxicity with secondary regenerative hyperplasia precedes, and is probably required for, hepatic and renal neoplasia; 2) there are no epidemiological data specific to chloroform and, at most, equivocal epidemiological data related to drinking water exposures that cannot necessarily be attributed to chloroform amongst multiple other disinfection byproducts; and 3) genotoxicity data on chloroform are essentially negative, although there are some scattered positive results that generally have limitations such as excessively high dose or with confounding factors. Thus, the weight-of-evidence of the genotoxicity data on chloroform supports a conclusion that chloroform is not strongly mutagenic, and that genotoxicity is not likely to be the predominant mode of action underlying the carcinogenic potential of chloroform. Although no cancer data exist for exposures via the dermal pathway, the weight-of-evidence conclusion is considered to be applicable to this pathway as well, because chloroform absorbed through the skin and into the blood is expected to be metabolized and to cause toxicity in much the same way as chloroform absorbed by other exposure routes.

For more detail on Characterization of Hazard and Dose Response, exit to the toxicological review, Section 6 (PDF).

For more detail on Susceptible Populations, exit to the toxicological review, Section 4.7 (PDF).

__II.A.2. Human Carcinogenicity Data

Inadequate. There are no epidemiological data attributing cancer to exposure to chloroform *per se*. Although there are some equivocal epidemiological data relating a weak association of drinking water exposures to bladder, rectal and colon cancer (Morris et al. 1992 ; McGeehin et al., 1993; Vena et al. 1993; Morris, 1995; King and Marrett, 1996; Doyle et al., 1997; Freedman et al., 1997; Cantor et al, 1998; Hildesheim et al., 1998), these studies can not attribute to chloroform among multiple other disinfection byproducts (DBPs) (SAB, 2000, ATSDR, 1997; IPCS, 2000). Morris et al. (1992) did a meta-analysis that pooled the relative risks from ten cancer epidemiology studies in which there was a presumed exposure to chlorinated water and its byproducts and estimated that approximately 10,000 cases of rectal and bladder cancer cases per year could be associated with exposure to DBPs in chlorinated water in the United States. Later, Poole (1997) reviewed the studies available to Morris et al. (1992) plus three additional studies (McGeehin et al., 1993; Vena et al., 1993; and King and Marrett, 1996). Poole (1997) observed that there

was considerable heterogeneity among the data and that there was evidence of publication bias within the body of literature. In addition, Poole found that the aggregate estimates reported by Morris et al. were sensitive to small changes in the analysis (e.g., addition or deletion of a single study). Based on the observations, Poole recommended that the cancer epidemiology data considered in the Morris evaluation should not be combined into a single summary estimate and that the data had limited utility for risk assessment purposes. Based on the available cancer epidemiology database, bladder cancer studies provide the strongest evidence for an association between exposure to chlorinated water and cancer. Based on the studies of Cantor et al. (1985), McGeehin et al. (1993), King and Marrett (1996), Freedman et al. (1997), and Cantor et al. (1998), EPA calculated that the population attributable risk (the fraction of a disease that could be eliminated if the exposure of concern were eliminated) for bladder cancer ranged from 2% to 17% (U.S. EPA, 1998c). However, these calculations are based on a number of assumptions, including the assumption that there is a cause-effect relationship between exposure to chlorinated drinking water and increased risk of bladder cancer. This assumption is subject to considerable uncertainty, especially because findings are not consistent within or between studies. Evaluation of these studies by application of standard criteria for establishing causality from epidemiological observations (strength of association, consistency of findings, specificity of association, temporal sequence, dose-response relation, biological plausibility) has led EPA to conclude that the current data are insufficient to establish a causal relationship between exposure to chloroform and increased risk of cancer (U.S. EPA, 1998a). Moreover, if, in the future, the weight-of-evidence does reach a point where a causal link is established between exposure to chlorinated water and increased risk of bladder or other types of cancer, it could not be concluded from epidemiological studies of this type that chloroform per se is carcinogenic in humans, as chlorinated water contains numerous disinfection byproducts besides chloroform that are potentially carcinogenic (U.S. EPA, 1998a).

__II.A.3. Animal Carcinogenicity Data

Adequate. At high doses, chloroform has been reported to be carcinogenic in several chronic animal bioassays, with significant increases in the incidence of liver tumors in male and female mice and significant increases in the incidence of kidney tumors in male rats and mice (U.S. EPA, 1994, 1998c). When examining the biology of the tumor production, the occurrence of tumors is demonstrably species-, strain-, and gender-specific, and has only been observed under dose conditions that caused cytotoxicity and regenerative cell proliferation in the target organ.

In a gavage bioassay (NCI, 1976), Osborne-Mendel rats and B6C3F1 mice were treated with chloroform in corn oil 5 times/week for 78 weeks (50 animals per sex per dose group). Male rats received 90 or 125 mg/kg/day; females initially were treated with 125 or 250 mg/kg/day for 22 weeks and 90 or 180 mg/kg/day thereafter. A decrease in survival rate and weight gain was evident for all treated rats. A significant increase in kidney epithelial tumors was observed in male rats (0% in controls, 8% in the low dose and 24% in the high dose groups). Male mice received 100 or 200 mg/kg/day, raised to 150 or 300 mg/kg/day at 18 weeks; females were dosed with 200 or 400 mg/kg/day, raised to 250 or 500 mg/kg/day. Survival rates and weight gains were comparable for all groups except high dose female mice which had a decreased survival. In mice, highly significant increases in hepatocellular carcinomas were observed in both sexes (98% and 95% for males and females at the high dose; 36% and 80% for males and females at the low dose as compared with 6% of both matched and colony control males , 0% in matched control females and 1% in colony control females). Nodular hyperplasia of the liver was observed in many low dose male mice that had not developed hepatocellular carcinoma. Hepatomas have also developed in female strain A mice and NLC mice gavaged with chloroform (Eschenbrenner and Miller, 1945; Rudali, 1967).

Jorgenson et al. (1985) administered chloroform (pesticide quality and distilled) in drinking water to male Osborne-Mendel rats and female B6C3F1 mice at

concentrations of 200, 400, 900, and 1,800 mg/L for 104 weeks. These concentrations were reported by the author to correspond to 19, 38, 81, and 160 mg/kg/day for rats and 34, 65, 130, and 263 mg/kg/day for mice. The combined benign and malignant renal tumor incidence in male rats was 2%, 2%, 2%, 5%, 6% and 14% for the control, matched control, 19, 38, 81, and 160 mg/kg/day groups, respectively. A significant increase in renal tumors (14%) in rats was observed in the highest dose group (160 mg/kg/day). A reevaluation of the histopathology of the slides (Hard et al., 2000), found evidence of persistent cytotoxicity and regenerative hyperplasia in all rats of the highest dose group. Similar changes were also observed in rats at 81 mg/kg/day, but at a much lower incidence and grade. Thus, the histopathology reexamination provides evidence supporting chronic renal tubule injury as the mode of action underlying the renal tumor response. The liver tumor incidence in female mice was not significantly increased.

Chloroform administered in toothpaste was not carcinogenic to male C57B1, CBA, CF-1, or female ICI mice or to beagle dogs. Male ICI mice administered 60 mg/kg/day were found to have an increased incidence of kidney epithelial tumors (Roe et al., 1979; Heywood et al., 1979). A pulmonary tumor bioassay in strain A/St mice was negative, as was one in which newborn C57X DBA2/F1 mice were treated s.c. on days 1 to 8 of life (Theiss et al., 1977; Roe et al., 1968).

Matsushima (1994) exposed F344 rats (50/sex/group) and BDF1 mice (50/sex/group) to chloroform vapor 6 hours/day, 5 days/week for 104 weeks. Rats were exposed to concentrations of 0, 10, 30, or 90 ppm, and mice were exposed to 0, 5, 30, or 90 ppm. In order to avoid short- term lethality, mice in the two highest groups (30 and 90 ppm) were initially exposed to a lower levels for 2-6 weeks before the long-term exposure. The time-weighted average (TWA) for the 30 ppm group was 29.1 ppm and for the 90 ppm group was 85.7 ppm (U.S. EPA, 1998a). Statistically significant increases in the incidence of overall renal cell adenoma and renal cell carcinoma were observed in male mice in the 30 (7/50) and 90 (12/48) ppm groups, when compared to controls (0/50). The overall incidence rates of renal cell carcinoma were statistically significantly increased in males in the 90-ppm group (11/48) when compared to controls (0/50). There were no statistically significant findings reported for female mice in any exposure groups.

__II.A.4. Supporting Data for Carcinogenicity

Mutagenicity

Many studies have investigated the mutagenic potential of chloroform. However, there are several reasons these studies must be reviewed carefully and interpreted cautiously. For example, chloroform is relatively volatile, so test systems not designed to prevent chloroform escape to the air may yield unreliable results. Earlier studies in which appropriate P450-based metabolic activation systems were absent are also likely to be unreliable. Further, some older studies that used ethanol as a solvent or preservative for chloroform may be confounded by formation of ethyl or diethyl carbonate, which are potent alkylating agents. Another important issue is that studies that focused on clastogenicity endpoints using excessively high doses may be confounded by severe cytotoxicity, causing lysosomal or other releases (Brusick, 1986).

In Vitro Studies

Two investigators reported DNA binding in studies with calf thymus DNA in the presence of exogenous activation (DiRenzo et al., 1982; Colacci et al., 1991). The study by DiRenzo et al. (1982) used ethanol as a solvent, suggesting that ethyl carbonate formation might be a problem. In the study by Colacci et al. (1991), addition of SKF-525A inhibited DNA binding, suggesting that binding was mediated by a cytochrome P-450 mediated pathway, as would be expected for chloroform. In

interpreting these studies, it is important to remember that cell-free systems may not always be a good model for intact cellular processes.

Gene mutation studies in *Salmonella typhimurium* and *E. coli* (Ames assay), including tests done under conditions designed to reduce evaporation, are mostly negative, with or without activation with microsomes from liver or kidney of rats or mice (Rapson et al., 1980; San Agustin and Lim-Sylianco, 1978; Van Abbe et al., 1982; Uehleke et al., 1977; Gocke et al., 1981; Roland-Arjona et al., 1991; Le Curieux et al., 1995; Kirkland et al., 1981; Simmon et al., 1977). However, four studies have showed positive results in bacteria. Varma et al. (1988) reported that chloroform caused mutagenicity in five strains of *S. typhimurium*, but the response was noted only at the lowest dose tested, and all higher doses were not different from control. This unusual pattern casts some doubt on these results. San Agustin and Lim-Sylianco (1997) reported that chloroform caused DNA damage in *Bacillus subtilis*, and Wecher and Scher (1982) reported that chloroform caused mutations in *Photobacterium phosphoreum*. However, neither study reported the exposure concentrations that caused these effects, so the relevance of these reports is uncertain. In addition, the studies by Varma et al. (1988) and Wecher and Scher (1982) each used ethanol as a diluent, raising the possibility that the positive effect might be related to ethyl carbonate formation rather than to chloroform. The majority of results reported for *S. typhimurium* and *E. coli* exposed to the vapor phase were also negative (Van Abbe et al., 1982; Pegram et al., 1997; Simmon, 1977; Sasaki et al., 1998). Pegram et al. (1997) reported that chloroform was weakly positive at vapor concentrations greater than 19,200 ppm (about 770 mg/L in the aqueous phase). Employing physiologically based pharmacokinetic models, the authors estimated the oral doses needed to produce the effect would exceed 2,000 mg/kg (approximately twice the LD50).

Tests of genotoxicity are also mainly negative in fungi (Gualandi, 1984; Mehta and von Borstel, 1981; Kassinova et al., 1981; Jagannath et al., 1981). However, chloroform was shown to induce intrachromosomal recombination in *Saccharomyces cerevisiae* at concentrations of 6,400 mg/L (Callen et al., 1980) or 750 mg/L (Brennan and Schiestl, 1998). In the Brennan and Schiestl study, addition of *N*-acetylcysteine reduced chloroform-induced toxicity and recombination, suggesting a free radical may have been involved. Chromosome malsegregation was also reported in *Aspergillus nidulans* (Crebelli et al., 1988), but only at concentrations above 1,600 mg/L. In all three of these positive studies, doses that caused positive results also caused cell death, indicating that exposures were directly toxic to the test cells.

Studies in intact mammalian cells are mainly negative (Larson et al., 1994a; Perocco and Prodi, 1981; Butterworth et al., 1989; Kirkland et al., 1981; White et al., 1979; Sturrock, 1977), although positive results have been reported in a few systems. Increased sister chromatid exchange was reported in human lymphocytes at a concentration of about 1,200 mg/L without exogenous activation (Morimoto and Koizumi, 1983), and at a lower concentration (12 mg/L) with exogenous activation (Sobti, 1984). In the study by Sobti, the increase was quite small (less than 50%), and there was an increase in the number of cells that did not exclude dye. This suggests that the exposure levels that caused the mutagenic effect may have been directly toxic to the cells. In addition, ethanol was used as a dose vehicle. Mitchell et al. (1988) did not detect an increase in mutation in mouse lymphoma cells at an exposure level of 2,100 mg/L in the absence of exogenous activation, but did detect an effect at a concentration of 59 mg/L with exogenous activation.

In Vivo Studies

A number of different endpoints of chloroform genotoxicity have been measured in intact animals exposed to chloroform either orally or by inhalation. In studies of DNA binding in liver and kidney of mice and rats, negative results have been reported at

doses of 742 mg/kg, 119 mg/kg, and 48 mg/kg (Diaz-Gomez and Castro, 1980; Reitz et al., 1982; Pereira et al., 1982). However, positive results have been reported at doses as low as 2.9 mg/kg (Colacci et al., 1991). But, in the study by Colacci et al. (1991), no significant difference in binding was noted between multiple tissues (liver, kidney, lung, and stomach), and there was no increase in binding with phenobarital pretreatment. This suggests the binding may not have been related to chloroform metabolism.

Studies based on signs of DNA damage or repair have been uniformly negative (Larson et al., 1994a; Potter et al., 1996; Reitz et al., 1982; Mirsalis et al., 1982). However, studies based on various signs of chromosomal abnormalities have been mixed, with some studies reporting negative findings at doses of 371 mg/kg and 800 mg/kg (Shelby and Witt, 1995; Topham, 1980), while other studies report positive results at doses as low as 1.2 mg/kg (Fujie et al., 1990). However, the positive result at low dose in the study by Fujie et al. (1990) was observed following intraperitoneal exposure, and positive results following oral exposure were not observed until a dose level of 119 mg/kg. Morimoto and Koizumi (1983) observed an increase in the frequency of sister chromatid exchange in bone marrow cells at a dose of 50 mg/kg/day, but at 200 mg/kg/day, all of the mice died. As discussed before, mutagenicity results observed following highly toxic doses may have been confounded by cytotoxic responses and should be viewed as being of uncertain relevance.

Several studies have reported negative findings for the micronucleus test in rats and mice (Gocke et al., 1981; Salamone et al., 1981; Le Curieux, 1995), but several other studies have detected positive results, mainly at exposure levels of 400-600 mg/kg (San Agustin and Lim-Sylianco, 1982; Robbiano et al., 1998; Sasaki et al., 1998; Shelby and Witt, 1995). This suggests that chloroform may be clastogenic, but it is important to note that these doses are well above the level that causes cytotoxicity in liver and kidney in most oral exposure studies in rodents.

Butterworth et al. (1998) did not detect an increase in mutation frequency in male mice exposed by inhalation at an exposure level of 90 ppm, even though this exposure did cause an increase in tumors in the study by Nagano et al. (1998). Increased incidence of sperm head abnormalities was reported in mice exposed at 400 ppm (Land et al., 1981), but was not observed in mice exposed to 371 mg/kg intraperitoneally (Topham, 1980).

In *Drosophila melanogaster* larvae exposed to chloroform vapor, gene mutation (Gocke et al., 1981) and mitotic recombination tests (Vogel and Nivard, 1993) were both negative. Grasshopper embryos (*Melanoplus sanguinipes*) did not display mitotic arrest at vapor concentrations of 30,000 ppm, but an effect was seen at 150,000 ppm (Liang et al., 1983). San Agustin and Lim-Syllianco (1981) reported a single positive and negative result for host- mediated mutagenicity in *Salmonella typhimurium*, but exposure levels were not reported in either case.

On the basis of the in vitro and in vivo studies reviewed above, even though a role of mutagenicity cannot be completely ruled out, the majority of available studies are negative, and many of the positive studies have limitations (excessive doses or other confounding factors). Thus, the weight-of-evidence supports the conclusion that chloroform is not strongly mutagenic, and that genotoxicity is not likely to be the predominant mode of action underlying the carcinogenic potential of chloroform. This conclusion is supported by a number of other groups who have reviewed and evaluated the available data on chloroform genotoxicity, including the International Commission for Protection against Environmental Mutagens and Carcinogens (Lohman et al., 1992), ILSI (1997), Health Canada (2000), and WHO (1998).

Mode of Action

1. Summary of Postulated Mode of Action

Studies in animals reveal that chloroform can cause an increased incidence of kidney tumors in male rats and an increased incidence of liver tumors in male and female mice. Available data suggest that tumors are produced only at dose levels that result in cytotoxicity. These induced tumor responses are postulated to be secondary to sustained or repeated cytotoxicity and secondary regenerative hyperplasia. Chloroform's carcinogenic effects in rodent liver and kidney are attributed to oxidative metabolism-mediated cytotoxicity in the target organs. Although chloroform undergoes both oxidative and reductive cytochrome P450-mediated metabolism, it is the oxidative (CYP2E1) metabolic pathway that predominates at low chloroform exposures. This oxidative pathway produces highly tissue-reactive metabolites (in particular phosgene) that lead to tissue injury and cell death. It is likely that the electrophilic metabolite phosgene causes cellular toxicity by reaction with tissue proteins and cellular macromolecules as well as phospholipids, glutathione, free cysteine, histidine, methionine, and tyrosine. The liver and kidney tumors induced by chloroform depend on persistent cytotoxic and regenerative cell proliferation responses. The persistent cell proliferation presumably would lead to higher probabilities of cell mutation and subsequent cancer. The weight of the evidence indicates that a mutagenic mode of action via DNA reactivity is not a significant component of the chloroform carcinogenic process.

2. Identification of key events

There are essentially three key steps in the sequence of events that lead to chloroform-induced tumorigenesis in the liver and kidneys of rodents. The first step is oxidative metabolism of chloroform in the target organs, kidney and liver. Numerous binding and metabolism studies (as described in ILSI, 1997, and U.S. EPA, 1998a) provide support that chloroform is metabolized by the oxidative cytochrome P450 (CYP2E1) pathway. This conclusion is supported by the study of Constan et al. (1999) in Sv/129 wild type, Sv/129 CYP2E1 null, and B6C3F1 mice. In the wild type of each strain, exposure to 90 ppm chloroform for 6 hours per day for 4 consecutive days resulted in severe hepatic and renal lesions along with increased cell proliferation. With the same exposure, neither the cytotoxicity nor cell proliferation occurred in the CYP2E1 null mouse or in the wild type of either strains treated with the P450 inhibitor ABT.

Available evidence indicates that metabolism by CYP2E1 predominates at low exposures and is rate-limiting to chloroform's carcinogenic potential. Reductive metabolism, if it occurs, can lead to free radicals and tissue damage, but this pathway is absent or minor under normal physiological conditions. The next key step is the resultant cytotoxicity and cell death caused by the oxidative metabolites (with phosgene as the significant toxic intermediate). Regenerative cell proliferation follows the hepatotoxicity and nephrotoxicity as measured by labeling index in mouse kidney and liver and rat kidney from chloroform-treated animals. This increase in cell division is responsible for the increased probability of cancer.

3. Strength, consistency, specificity of association

There are numerous cases where exposure to chloroform causes an increase in cytotoxicity (as evidenced by histopathological evaluation and/or increased labeling index) without any observable increase in cancer incidence. These data indicate that chloroform exposures that are adequate to cause cytotoxicity and regenerative cell proliferation do not always lead to cancer. However, there are no cases where a tumorogenic response has been observed in which evidence of cell regeneration is not also observed at the same or lower dose as that which caused an increase in tumors. This consistency of evidence (i.e., cell regeneration is detected in all cases of tumorigenicity) is strong evidence supporting the conclusion that cell regeneration is a mandatory precursor for tumorigenicity.

Evidence for a link between sustained cytotoxicity/regenerative hyperplasia and cancer is strongest in the kidney. In male Osborne-Mendel rats exposed to chloroform in water for 2 years (Jorgenson et al., 1985), a statistically significant increase in renal tumors was observed at a concentration of 1,800 ppm (160 mg/kg/day). A re-analysis of the histopathological slides from this study (Hard et al., 2000) revealed evidence for sustained cytotoxicity and cell proliferation in the kidney at exposures of 900 ppm (81 mg/kg/day) or higher. Likewise, in BDF_1 mice exposed to chloroform by inhalation at 5, 30, or 90 ppm for 6 hours/day, 5 days/week (Nagano et al., 1998), increased incidence of renal tumors was observed in male mice at the two higher doses, whereas females showed no significant tumor response. Templin et al. (1998) duplicated this exposure regimen in order to study whether the treatment caused cytotoxicity and regenerative hyperplasia. These authors observed cytotoxicity and hyperplasia in the kidneys of male mice exposed to 30 or 90 ppm throughout a 90-day exposure period, but not in females. This observation is consistent with the hypothesis that sustained cytotoxicity and regenerative hyperplasia are key events in the neoplastic response of the kidney to chloroform.

Available data also indicate that cytotoxicity and regenerative hyperplasia are required for liver cancer, although the strength of this conclusion is somewhat limited because most of the observations are based on short-term rather than long-term histological or labeling index measurements. For example, in the B6C3F1 mouse, corn oil gavage (bolus dosing) at the same doses that resulted in liver tumors in the study by NCI (1976) also caused hepatic cytolethality and a cell proliferative response at both 4 days and 3 weeks (Larson et al., 1994b,c). Similarly, exposure of female B6C3F1 mice to chloroform in drinking water at levels that did not induce liver tumors (Jorgenson et al., 1985) also did not induce hepatic cytolethality or cell proliferation at 4 days or 3 weeks (Larson et al., 1994b). This consistency of the data (i.e., evidence of cytolethality and/or regenerative hyperplasia is always observed in cases of increased liver tumors) supports the conclusion that this liver cancer also occurs via a mode of action involving regenerative hyperplasia.

4. Dose-response relationship

Chloroform-induced liver tumors in mice are only seen after bolus corn oil dosing. Mouse liver tumors are not found following administration by other routes (drinking water and inhalation). Rat liver tumors are not induced by chloroform following either drinking water or corn oil gavage administration. Kidney tumors are found in mice exposed to chloroform via inhalation or toothpaste preparations, and in rats when exposed via drinking water or corn oil gavage. Kidney and liver tumors develop only at doses that cause persistent cytotoxicity and regenerative proliferation, regardless of route of exposure or dosing regime. The overall dose-response for the cytotoxicity and cell proliferation responses is nonlinear. All key events and tumor effects depend on the dose-rate as shown by the difference in oil gavage versus drinking water administration (ILSI, 1997; U.S. EPA, 1998a).

5. Temporal relationship

As noted above, there is very strong evidence from short-term and long-term histological and labeling index studies in mice and rats that cytotoxicity and cell proliferation always precede the occurrence of increased kidney or liver tumor effects in long-term bioassays. For example, a re-evaluation of serial sacrifice data from the chloroform 2-year drinking water bioassay in Osborne-Mendel rats revealed a linkage between toxicity in the renal tubules and tumor development and showed that renal toxicity preceded tumor development (Hard and Wolf, 1999; Hard et al., 2000).

6. Biological plausibility and coherence

The theory that sustained cell proliferation to replace cells killed by toxicity, viral, or other insults such as physical abrasion of tissues can be a significant risk factor for

cancer is plausible and generally accepted (Correa, 1996). It is logical to deduce that sustained cytotoxicity and regenerative cell proliferation may result in a greater likelihood of mutations being perpetuated with the possibility of more of these resulting in uncontrolled growth. It may also be that continuous stimulus of proliferation by growth factors involved in inflammatory responses increases the probability that damaged cells may slip through cell cycle check points carrying DNA alterations that would otherwise be repaired. Current views of cancer processes support both these possibilities. There are no data on chloroform that allow the events that occur during cell proliferation to be directly observed. A high proliferation rate alone is not assumed to cause cancer; tissues with naturally high rates of turnover do not necessarily have high rates of cancer and tissue toxicity in animal studies does not invariably lead to cancer. Nevertheless, regenerative proliferation associated with persistent cytotoxicity appears to be a risk factor of consequence.

7. Role of genotoxicity

As noted above, the question whether chloroform or a metabolite is mutagenic has been tested extensively across different phylogenetic orders (i.e., bacterial, eukaryotic, and mammalian systems). Predominately negative results are reported in all test systems, with no pattern of mutagenicity seen in any one system considered to be a competent predictor. Positive results appear sporadically in the database, but they generally have problems with high dose or other confounding issues. ILSI (1997) considered results from 40 tests by the quantitative weight-of-evidence method for heterogeneous genetic toxicology databases from the International Commission for Protection against Environmental Mutagens and Carcinogens (ICEMC) (Lohman et al., 1992). This method scores relative DNA reactivity, with a maximum positive score being +100 and maximum negative -100. The maximum positive score obtained among 100 chemical databases has been +49.7 (triazaquone) and the maximum negative has been -27.7. The score for chloroform was -14.3.

Testing of chloroform in the p53 heterozygous knockout mouse shows no tumor effect (Gollapudi et al., 1999). Heterozygous p53 males were dosed up to 140 mg/kg and females up to 240 mg/kg via corn oil gavage for 13 weeks. This model is known to respond most effectively to mutagenic carcinogens.

Products of oxidative and reductive metabolism of chloroform are highly reactive. Such species are unstable and will likely react with cytoplasmic molecules before reaching nuclear DNA. Such reactive species (e.g., phosgene) have not been evaluated separately for genetic toxicity, and because of reactivity, would not be amenable to study and would not likely be able to transport from the cellular site of production to the nucleus.

Comparative examination of both oxidative and reductive metabolism for structural analogues and chloroform has revealed that carbon tetrachloride, which is largely metabolized to a free radical via the reductive pathway, results in cell toxicity, not mutagenicity. Moreover, chloroform and carbon tetrachloride show very different patterns of liver toxicity (i.e., carbon tetrachloride's toxicity is more consistent with free radical production and chloroform's is not). For methylene chloride, glutathione conjugation results in mutagenic metabolites. When rat glutathione transferase gene copies are introduced into *Salmonella*, bromodichloromethane produces mutagenic metabolites; the fact that chloroform in this system did so only marginally and only at high toxic doses (Pegram et al., 1997) supports a conclusion that the reductive pathway does not contribute to chloroform's toxicity or carcinogenicity.

In initiation-promotion studies, chloroform at the highest test dose of the drinking water bioassay does not promote development of hepatic lesions in rats or two strains of mice, nor does it initiate or act as a cocarcinogen. Administered in oil, chloroform was a promoter in the rat liver in initiation-promotion protocols. These

results are more consistent with the postulated mode of action than with any mutagenic potential.

8. Effects on children

The central questions asked in a mode of action analysis are, 1) whether the standard assumption that a mode of action observed in animals is relevant to humans holds true in a particular case, and 2) what the nature of the mode of action implies about the shape of the dose response relationship. In the case of chloroform the conclusions have been that the rodent mode of action can be assumed to be relevant to humans and that a nonlinear approach is most appropriate. The next question is whether the data lead one to anticipate similarities or differences in response by sex or age.

Ideally, one would have adequate data to compare each of the key events of chloroform toxicity and subsequent carcinogenicity in tissues of adults with those of the developing fetus and young. This kind of information is currently not to be found. In the absence of data on the fetus and young specific to chloroform, an evaluation is made as to whether a cogent biological rationale exists for determining that the postulated mode of action is applicable to children (EPA, 1999). There is no suggestion from available studies of chloroform to indicate that children or fetuses would be qualitatively more sensitive to its effects than adults. The developing organism would not be expected to be particularly sensitive to cytotoxic agents at minimally toxic levels because cell division is proceeding rapidly and repair capacity at the molecular and cellular level is high. This is reflected by the relatively low incidence of spontaneous tumors in developing and young organisms. Moreover, the reproductive and developmental studies available, while they have limitations, show that fetal effects are seen only at doses at which maternal toxicity is evident. Research would be needed to further explore whether there are circumstances in which this relationship does not hold. Research would also be needed to discover whether there is some other mode of action, not seen in rodents, that might be possible. Presently, there are no clues from in vivo or in vitro studies as to what alternative mode of action might be considered. In keeping with traditional toxicologic evaluations, chloroform has been tested in lifetime studies with high level doses to provide maximal opportunities for toxicologic effects to manifest themselves in multiple tissues and organs through multiple mechanisms. In the absence of data to the contrary, this approach is considered to provide evidence for lack of potential for significant response, other than those noted, even for sensitive individuals and life stages.

The mode of action analyzed as well as all other potential modes of action identified required that chloroform be metabolized by cytochrome P450 (CYP2E1) (SAB (2000), p.2). When this is considered along with the comparison of this enzyme activity between adults and the young there is confidence in assuming similarity in response among life stages. Further research on the processes of cell injury, death and regeneration would increase this confidence by addressing any uncertainty about potential quantitative similarity. The literature does not reveal any such quantitative data at present.

Given the above, it is reasonable to assume that: 1) The reactive metabolite inside the cell should have similar effects by reacting with and disrupting macromolecules in the cells of fetuses, children and adults, 2) Cell necrosis and reparative replication are not likely to be qualitatively different in various stages in life, 3) Cancer risk to the fetus or children would be a function of cytotoxic injury, like in adults, and protecting these life stages from sufficient cytotoxicity to elicit this response should protect against cancer risks. Further research would be needed to assess whether there are significant quantitative differences between life stages which have not yet been elucidated.

It can be noted that if data indicated that it were appropriate to apply a linear approach to part of a lifetime, such as the first 3 years of life, the resulting risk would be represented by a small increment of the total dose per body weight over a lifetime since most of a 70 year life is at an adult body weight. When this total is divided by 70 years to derive the lifetime average daily dose, the small increment of early dose does not significantly increase risk.

9. Conclusion regarding cancer mode of action

The weight of the evidence supports the conclusion that chloroform-induced tumors in liver and kidney are produced only at dose levels that result in repeated or sustained cytotoxicity and regenerative cell proliferation. A wide range of evidence across different species, sexes, and routes of exposure implicates oxidative CYP2E1 metabolism leading to persistent cytotoxicity and regenerative cell proliferation as events that precede and are associated with tumor formation. The cytochrome P450 oxidative metabolism that leads to oxidative damage and ensuing cell growth, involving basic tissue responses to cellular toxicity and death, is common to humans and rodents. No data exist indicating that the mode of action observed in rodents is not also likely to apply to humans.

Available data on the mutagenic potential of chloroform are mixed, but the majority of tests are negative, and some of the positive results are observed only at extreme exposure conditions. Thus, the weight of the evidence indicates that chloroform is not a strong mutagen and that neither chloroform nor its metabolites readily bind to DNA. On the basis of these results and the results of studies that evaluated other endpoints of mutagenicity, it seems likely that even though a role for mutagenicity cannot be excluded with certainty, chloroform does not produce carcinogenic effects primarily by a specific genotoxic mechanism.

The proposed dose-response relationship for chloroform tumorigenesis by the cytotoxicity-regenerative hyperplasia mode of action will be nonlinear, as it is dependent on biochemical and histopathological events that are nonlinear. The dose-response assessment would ideally be based on use of phosgene dosimetry because it marks the rate-limiting step of oxidative metabolism. The toxicokinetic modeling to support this phosgene approach is not currently available, so the dose-response assessment is based on the tumor precursor event of cytotoxicity to project a level of exposure that will be protective against the key event of regenerative hyperplasia.

Back to top

_II.B. Quantitative Estimate of Carcinogenic Risk from Oral Exposure

In accord with proposed EPA guidelines for cancer risk assessment (U.S. EPA, 1996), the method used to characterize and quantify cancer risk from a chemical depends on what is known about the mode of action of carcinogenicity and the shape of the cancer dose-response curve for that chemical. A default assumption of linearity is appropriate when evidence supports a mode of action of gene mutation due to DNA reactivity, or another mode of action that is anticipated to be linear. The linear approach is used as a matter of policy if the mode of action of carcinogenicity is not understood. Alternatively, an assumption of nonlinearity is appropriate when there is no evidence for linearity and sufficient evidence to support an assumption of nonlinearity. In this case, the carcinogenicity may be a secondary effect of toxicity that itself is a threshold phenomenon (U.S. EPA, 1996).

In the case of chloroform, the mode of action of carcinogenicity is reasonably well understood. Available data indicate that chloroform is not strongly mutagenic and chloroform is not expected to produce rodent tumors via a mutagenic mode of action (ILSI, 1997). Rather, there is good evidence that carcinogenic responses observed in animals are associated with regenerative hyperplasia that occurs in response to cytolethality (ILSI, 1997; U.S. EPA, 1998a,b). Because cytolethality occurs only at exposure levels above some critical dose level, a nonlinear approach is considered the most appropriate method for characterizing the cancer risk from chloroform.

The Proposed Guidelines for Carcinogenic Risk Assessment (U.S. EPA, 1996) state that when the mode-of-action analysis based on available data indicates that "the carcinogenic response is secondary to another toxicity that has a threshold, the margin-of-exposure analysis performed for toxicity is the same as is done for a noncancer endpoint, and an RfD for that toxicity may be considered in the cancer assessment." For chloroform, available evidence indicates that chloroform-induced carcinogenicity is secondary to cytotoxicity and regenerative hyperplasia; hence, the Agency relies on a nonlinear dose-response approach and the use of a margin-of-exposure analysis for cancer risk. The Agency has also chosen not to rely on a mathematical model to estimate a point of departure for cancer risk estimate, because the mode of action indicates that cytotoxicity is the critical effect and the reference dose value is considered protective for this effect.

RfD and Margin of Exposure

For more discussion of margin of exposure (MOE), see the Toxicological Review for Chloroform. Based on the kidney tumor of the drinking water study (Jorgenson et al., 1985), a point of departure (Pdp or LED_{10}) of 23 mg/kg/day can be calculated using quantitative modeling of tumor dose-response data. Comparing the Pdp to the RfD of 0.01 mg/kg/day leads to a MOE of 2,000, which is considered large. Thus, in this case, the RfD for noncancer effect is also considered adequately protective of public health for cancer effects by the oral route, on the basis of the nonlinear dose response for chloroform and the mode of action for both cancer and noncancer effects having a common link through cytotoxicity.

As discussed above, the RfD for noncancer effects is derived from the most sensitive endpoint in the most sensitive species. The RfD is based on fatty cysts formation (fat accumulation) in the liver and elevation of SGPT in dogs (Heywood et al., 1979). Hepatic fat accumulation and elevated SGPT are considered early signs of impaired liver function resulting from chloroform-induced cytotoxicity. This effect occurs at doses at or below those that cause increased labeling index, morphological changes, or cellular necrosis, so protection against this effect is believed to protect against cytolethality and regenerative hyperplasia. Accordingly, the RfD of 0.01 mg/kg/day presented in Section I.A.1 can be considered protective against increased risk of cancer.

__II.B.1. Summary of Risk Estimates

A dose of 0.01 mg/kg/day (equal to the RfD) can be considered protective against cancer risk

____II.B.1.1. Oral Slope Factor -- Not applicable (see text).

____II.B.1.2. Drinking Water Unit Risk -- Not applicable (see text).

__II.B.2. Dose-Response Data (Carcinogenicity, Oral Exposure)

Dose-response data used to derive the RfD for chloroform are presented in Section I.A.2.

__II.B.3. Additional Comments (Carcinogenicity, Oral Exposure)

Because chloroform is a volatile chemical, exposure to chloroform in drinking water may occur not only via direct ingestion, but also by inhalation of chloroform released from household uses of water (showering, cooking, washing, etc.) into indoor air. Therefore, assessments of cancer and noncancer health effects from chloroform in water should account for exposures by all pathways, including oral, inhalation, and dermal.

__II.B.4. Discussion of Confidence (Carcinogenicity, Oral Exposure)

Confidence in the cancer assessment for chloroform is rated as medium. This is based on a strong database in animals that supports the conclusion that cancer does not occur without antecedent cytotoxicity and regenerative hyperplasia, leading in turn to the conclusion that cancer risk is negligible at doses that do not result in cytotoxicity. Confidence in this conclusion is tempered by absence of direct studies in humans and by the finding that there are some positive results in studies on the mutagenicity of chloroform, even though the weight-of-evidence indicates that chloroform is not a strong mutagen and that a mutagenic mode of action is not likely to account for the cancer responses observed in animals.

EPA is currently revising its guidelines for cancer risk assessment. Among other issues, EPA is looking closely at how to assess whether a postulated mode of action in adults is applicable to children. When the guidelines are final, EPA will consider their impact on existing health assessments on IRIS.

Back to top

_II.C. Quantitative Estimate of Carcinogenic Risk from Inhalation Exposure

NOTE: The following evaluation of cancer risk from chloroform inhalation was developed in 1987 and does not incorporate newer data or the 1996 or 1999 draft cancer assessment guidelines. EPA is currently working to revise the assessment for inhalation exposure.

__II.C.1. Summary of Risk Estimates

____II.C.1.1. Inhalation Unit Risk -- 2.3E-5 per (ug/m3).

____II.C.1.2. Extrapolation Method -- Linearized multistage procedure, extra risk.

Air Concentrations at Specified Risk Levels:

Risk Level	Concentration
E-4 (1 in 10,000)	4E+0 µg/m3
E-5 (1 in 100,000)	4E-1 µg/m3
E-6 (1 in 1,000,000)	4E-2 µg/m3

__II.C.2. Dose-Response Data for Carcinogenicity, Inhalation Exposure

Tumor Type -- hepatocellular carcinoma
Test Animals -- mouse, B6C3F1, female
Route -- oral, gavage
Reference -- NCI, 1976

Dose		
Administered (mg/kg/day)	Human Equivalent (mg/kg/day)	Tumor Incidence
Female		
0	0	0/20
238	9.9	36/45
477	19.9	39/41
Male		
0	0	1/18
138	6.2	18/50
277	12.5	44/45

__II.C.3. Additional Comments (Carcinogenicity, Inhalation Exposure)

This inhalation quantitative risk estimate is based on data from a gavage study. Above doses are TWA; body weights at the end of the assay were 35 g for males and 28 g for females. Vehicle control animals were run concurrently and housed with test animals. All treated animals experienced decreased body weight gain. Survival was reduced in high-dose males and in all treated females. Experimental data for this compound support complete absorption of orally administered chloroform under conditions of this assay. There are no apparent species differences in this regard. Extrapolation of metabolism-dependent carcinogenic responses from mice to humans on the basis of body surface area is supported by experimental data. The incidence data for both male and female mice were used to derive slope factors of 3.3E-2 and 2.0E-1 per (mg/kg)/day, respectively. The unit risk was prepared by taking a geometric mean of the slope factor and assuming 100% for low doses of chloroform in air. The unit risk should not be used if the air concentration exceeds 400 $\mu g/m^3$, because above this concentration the unit risk may not be appropriate.

__II.C.4. Discussion of Confidence (Carcinogenicity, Inhalation Exposure)

Adequate numbers of animals were treated and observed. Risk estimates derived from male rat kidney tumor data (2.4E-2) (NCI, 1976) and studies by Roe et al. (1979) (1.0E-1) are generally supportive of the risk estimate.

Back to top

_II.D. EPA Documentation, Review, and Contacts (Carcinogenicity Assessment)

__II.D.1. EPA Documentation

Source Document -- U.S. EPA, 2001 (oral carcinogenicity assessment); U.S. EPA, 1985, 1987 (inhalation carcinogenicity assessment)

This assessment was peer reviewed by external scientists. Their comments have been evaluated carefully and incorporated in finalization of this IRIS summary. A

record of comments on the oral carcinogenicity assessment is included in an appendix to U.S. EPA (2001). ***To review this appendix, exit to the toxicological review, Appendix A, External Peer Review -- Summary of Comments and Disposition (PDF)***.

__II.D.2. EPA Review (Carcinogenicity Assessment)

Agency Consensus Date (oral carcinogenicity assessment) -- 7/27/2001
Verification Date (inhalation carcinogenicity assessment) – 8/26/1987

__II.D.3. EPA Contacts (Carcinogenicity Assessment)

Please contact the IRIS Hotline for all questions concerning this assessment or IRIS, in general, at (202)566-1676 (phone), (202)566-1749 (FAX) or hotline.iris@epa.gov (internet address).

Back to top

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_VI. Bibliography

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Chloroform
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Back to top

_VI.B. Inhalation RfC References

(Not applicable.)

Back to top

_VI.C. Carcinogenicity Assessment References

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Back to top

_VII. Revision History

Chloroform
CASRN -- 67-66-3

Date	Section	Description
03/01/1988	I.A.1.	Dose conversion clarified
03/01/1988	I.A.2.	LOAEL and RfD in text corrected
03/01/1988	I.A.4.	Text revised
03/01/1988	I.A.5.	Text revised
06/30/1988	II.	Carcinogen summary on-line
06/30/1988	I.A.7.	Primary contact changed
10/01/1989	I.B.	Inhalation RfD now under review
06/01/1990	IV.A.1.	Area code for EPA contact corrected
06/01/1990	IV.F.1.	EPA contact changed
01/01/1991	II.	Text edited
01/01/1991	II.C.1.	Inhalation slope factor removed (global change)
02/01/1991	II.C.3.	Information on extrapolation process included
02/01/1991	II.C.4.	Text edited
03/01/1991	II.D.3.	Primary contact changed
01/01/1992	IV.	Regulatory actions updated
04/01/1992	IV.A.1.	CAA regulatory action withdrawn
07/01/1992	I.A.	Clarify Schwetz citation
07/01/1992	VI.C.	Oral RfD references on-line
07/01/1992	VI.C.	Carcinogenicity assessment references on-line
09/01/1992	I.A.7.	Primary contact changed
08/01/1995	I.B.	EPA's RfD/RfC and CRAVE workgroups were discontinued in May, 1995. Chemical substance reviews that were not completed by September 1995 were taken out of IRIS review. The IRIS Pilot Program replaced the workgroup functions beginning in September, 1995.
04/01/1997	III., IV., V.	Drinking Water Health Advisories, EPA

		Regulatory Actions, and Supplementary Data were removed from IRIS on or before April 1997. IRIS users were directed to the appropriate EPA Program Offices for this information.
12/10/1998	I.B.	This chemical is being reassessed under the IRIS Program.
10/19/2001	I.A.,VI	Oral RfD and references updated
10/19/2001	II.B.,VI	Oral carcinogenicty assessment and references updated
01/08/2002	II.C.1.	Corrected typographical error in units in inhalation unit risk.
03/26/2002	Tox. Review	Corrected list of external peer reviewers.

Back to top

_VIII. Synonyms

Chloroform
CASRN -- 67-66-3
Last Revised -- 10/19/01

67-66-3
Chloroform
Formyl Trichloride
Freon 20
Methane Trichloride
Methane, Trichloro-
Methenyl Chloride
Methenyl Trichloride
Methyl Trichloride
NCI-CO2686
R-20
TCM
Trichloroform
Trichloromethane

Note: A TOXICOLOGICAL REVIEW is available for this chemical in Adobe* PDF format (112 Pages, 760 Kbytes). Similar documents can be found in the List of Available IRIS Toxicological Reviews.

* You will need Adobe Acrobat Reader, available as a free download, to view some of the files on this page. See EPA's PDF page to learn more about PDF, and for a link to the free Acrobat Reader.

Back to top
